Features of the scattering of polarized light by biological materials of fish

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ABSTRACT

The article presents the results of a study of the regularities of the scattering of polarized light by fish mucus and fish scales in order to identify matrix elements that are most sensitive to changes in the conformation, shape and size of protein macromolecules of fish mucus, and to identify the structural features of fish scales.

Keywords: polarized light, fish mucus, fish scales

1. INTRODUCTION

Scaly formations are characteristic of the integument of representatives of various classes of chordates. The accumulation of a huge amount of factual material of scales both from recent species and from paleontological finds requires the development of methodological approaches to their systematization, including using modern optical methods.

Elasmoid scales of teleost fish are especially interesting from the point of view of studying optical effects. This type of scales is formed by bone tissue, however, unlike other groups of vertebrates, the thickness of the mature bone tissue of teleost fish does not contain living osteocytes - cells that provide the synthesis of the main elements of the bone matrix. This type of bone is called anosteocytic. The formation and growth of morphological structures, including scales, from this type of tissue is possible due to the fact that each subsequent layer of osteoblasts develops from osteogenic cells only on the surface of an already formed bone element. As new layers of bone matrix are layered, osteoblasts move to the periphery of the structure, without going deep into its depths. Thus, the bone matrix of scaly plates is monolithic, while the bone structures of other vertebrates are penetrated by numerous cavities in the form of lacunae of osteocytes and mijlacunar tubules.

In terms of chemical composition, elasmoid scales are a multicomponent system, in which the proportion of minerals, depending on the species of fish, ranges from 16 to 59%, and the organic component is mainly represented by fibrillar proteins. Among the latter, about 24% is collagen, and 76% is ichtylepidin, a protein that in its properties occupies an intermediate position between collagen and keratin. This protein remains in the scales even after collagen and calcium ions are extracted from it.

The scale mineralization is variable. The content of mineral salts can vary significantly in different areas. Also, the degree of mineralization is influenced by the physiological state, for example, in sexually mature females during the maturation of eggs, the concentration of calcium ions in the blood and ovaries significantly increases due to its washing out of the scales, manifests itself in the destruction of individual elements of the relief of the bone plate of the scale - the so-called sclerites. Scleritis is a thickening of the bone plate, which is located concentrically around its perimeter. As the fish grows, the radius of the scales and its thickness increase due to the laying of a new bone plate with a large diameter in the already formed scales, and its free peripheral portion thickens, enveloping the previously formed bone plate on the sides.

In the general morphology of elasmoid scales, the following zones can be distinguished: cranial field (anterior field) - the anterior part of the scales, immersed in Corum; caudal field (posterior field) - posterior free edge of the scales; dorsal field ventral field. Usually, the cranial, dorsal and ventral fields are covered by anterior scales. These three areas form an imbedded portion. In addition, the cranial field is immersed in the depths of the corium, in the so-called skin pocket. The caudal field forms an exposed portion. There is a noticeable epidermis in this area of uncleaned DOPR scales.

The place where the scales start to form is the scale center. In this area, the greatest thickness of the scaly plate.

Fish mucus contains various components, in particular proteins (60-70% dry weight), carbohydrates, lipids (3-5%), as well as some metabolites.

Mucins form the basis of mucus - these are high molecular weight glycoproteins that provide mucus with viscoelastic and rheological properties. In fish mucus, they can be represented by neutral glycoproteins, but they are often acidified with sialic acid (carboxylated with monosaccharide) or sulfated with monosaccharides. Mucins are usually repetitive sites rich in threonine, serine and proline (Rose and Voynow, 2006).

Mucins are polymers that have polydisperse sizes (1-10 microns in length) and mass (2-50 MDA). Glycosaminoglycans account for 70-80% of the mucin mass (McGuckin et al., 2015).

Proteases, antimicrobial peptides, lectins, lysozyme, immunoglobulins, complement proteins, C-reactive protein, transferrin, and other antibacterial proteins and peptides are recorded in mucus (Shoemaker et al., 2005; Swain et al. , 2007). It is known that most functional proteins (enzymes, immunoglobulins) have a globular structure. The polypeptide chain of a globular protein with a length of 50-150 (rarely up to 250) amino acid residues forms a whole globule 25-40 Å in size. Protein globules are represented by a hydrophobic core with non-polar amino acids. Externally - a shell with predominantly hydrophilic amino acids that come into contact with water and provide the formation of a hydration shell. The organization of the spatial structure of the globular protein through the formation of a strictly hydrophobic nucleus and mosaic surface limits the size of the globule, therefore, starting from about a molecular weight of 14-16 kDa, the polypeptide chain forms 2 or more globules with their separate hydrophobic nuclei - domains (Marchenko et al., 2012)

Fish mucus also contains a certain amount of carbohydrate components. Although their functions are not clearly defined, they are likely to have a protective role (Esteban, 2012).

Fish cutaneous mucus is reported to contain various saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). SFA, present in mucus - palmitic acid and stearic acid, MUFA - oleic acid, PUFA - linoleic, alpha-linolenic and moroctic acids. These fatty acids are thought to play an important role in protecting against pathogens (Balasubramanian and Gunasekaran, 2015).

Fish skin mucus also contains small amounts of metabolites and has antibacterial properties such as azelaic acid, N-acetylneuraminic acid and N-acetylglucosamine and hydroxyisocaproic acid (Ekman et al., 2015).

The composition of mucus is different for different fish species. The mucus storage is influenced by various endogenous (eg, sex, stage of development) and exogenous (eg, stress, hyperosmolarity, pH, and infections) (Ellis, 2001). In some cases, especially in injured fish, the protein content of the mucus increases. The epidermis of these fish produces a gel-like material that sticks to the skin, even if they swim at different speeds and for several days (Esteban, 2012)

2. EXPERIMENTAL EQUIPMENT

The measurements were carried out on a laboratory Stokes-goniometer, the schematic diagram of which is shown in Fig. 1. A continuous laser diode with a radiation wavelength λ = 670 nm was used as a source of monochromatic light. With the help of the illuminator, sight 2, the output beam of the laser diode is converted into a parallel light beam with a diameter of D ~ 2 mm and an aperture of $2\gamma = 30'$. Swiveling mirrors 3 with external coating make it possible to reduce the longitudinal dimensions of the Stokes-goniometer. For controlled attenuation, a block of neutral filters 4 with known attenuation coefficients is used. The system of polaroid and quarter-wave plate 5 converts the linearly polarized light of the laser diode (1) into circularly polarized, which provides light beams with different shapes and azimuths of polarization during the experiment. The polarizing device 6 consists of a linear polaroid that rotates in a frame and provides a linearly polarized beam with the required polarization azimuth, and in combination with λ / 4 - circularly polarized radiation. The test sample 8 is placed in a circular frame on the stage at the geometric center of rotation of the goniometric unit 7.The rotary arm of the goniometer is equipped with a receiving system, which consists of an analyzer 9 (λ / 4 plate and polaroid) and a receiver-sight 10, which provides the reception of optical radiation in aperture angle $2\gamma = 30'$. To receive weak streams of light scattered by the samples, an FEU-106 photomultiplier tube is used, which was powered by a high-voltage power supply unit 14, and the photocurrent was measured using a digital ampere-voltmeter. In other cases, photodiode FD-288A (11) with a constant signal amplifier (12). The entire receiving device rotated around the geometric center of the goniometric unit 7 in the horizontal plane by 175 ° with fixation every 5 °. The test specimens were adjusted relative to the geometric center of rotation of the goniometer using linear displacements of the stage in three mutually perpendicular planes, including vertical displacements. In addition, a mount can be placed on the stage for studying plane isotropically and anisotropically scattering objects, which allows you to change the spatial orientation of the object (Fig. 2).

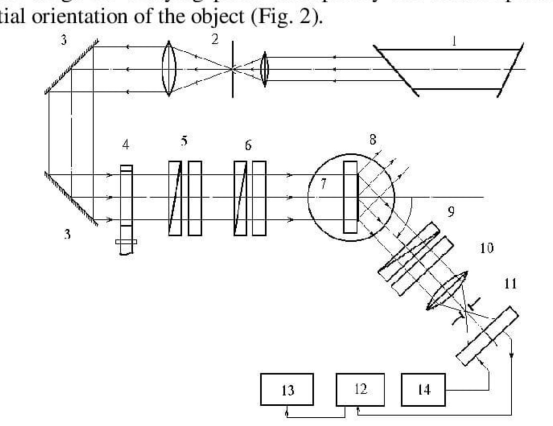

Fig. 1. Schematic diagram of a laboratory Stokes-goniometer

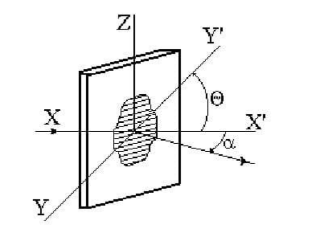

Fig. 2. Object location scheme

3. EXPERIMENT

The methods of optics of light-scattering media have found wide application for the study of dispersed media of biological origin in order to reveal the influence of the shape, orientational structure, and polydispersity of the components of a given medium on its optical characteristics.

A comprehensive study of the characteristics of light scattering and absorption makes it possible to identify physiological and morphological changes in cells caused by temperature and chemical factors, and to carry out a quick qualitative analysis. A significant number of biological structures undergo continuous conformational changes due to high physiological activity. Moreover, the time dependence of such structural transformations provides important information about the fine structure of cell formations during time. Taking into account the fact that the light scattering matrix contains all the information on the dispersed medium available by optical methods, it is advisable to use the Mueller matrix method to study conformational changes.

This section presents the results of studies of aqueous solutions of fish mucus in order to identify conformational transformations under the influence of temperature.

We carried out studies of the MRS of fish mucus solutions when the temperature changed in the range of 20 °¸60 ° C. The solutions were placed in a rectangular cuvette with the possibility of thermostating for the period of measurements.

As a result of the studies carried out, the following features of the behavior of nonzero components of the MRS of such aqueous solutions of fish mucus can be distinguished, depending on the temperature change at scattering angles $\alpha_1 = 0°$ and $\alpha_2 = 90°$.

1. First of all, it should be emphasized that when conducting research, the components $f_{12}(f_{21})$ at different temperatures at $\alpha = 0°$ do not take positive values (the effects of absorption by mucus macromolecules are insignificantly expressed). With an increase in the temperature of the saline solution of fish mucus from 22 ° C to 60 ° C, the component $f_{12}(f_{21})$ (Fig. 3, curve 1) changes in absolute value from 0.82 at 22 ° C to 0.23 at 60 ° C, confirms the previous theoretical assumption about the gradual denaturation of fish mucus. A further decrease in the component $f_{12}(f_{21})$ is associated with an increase in the scattering multiplicity, which leads to a decrease in the absolute value of all polarization components of the matrix.

2. Deviation of the component f_{22} fig. 4 from a single value is unambiguously associated with the difference in the shape of the scattering centers of the medium from spherical and the depolarization factor D = $(1 - f_{22})$ and is a measure of the asphericity of the particles, and with an increase in the dimension of the particle parameter, the factor D should change insignificantly.

3. In our case, the difference in the value D = $(1 - f_{22})$ at $\alpha \sim 90°$ scada for fish mucus is about 20-30%, which indicates a slight deviation of the macromolecule shape from spherical. With an increase in temperature, and hence a change in the shape (due to the transition to a spiral state) of the macromolecule, we observe a change in the value of the component f_{22} in absolute value from 0.89 to 0.56, which indicates a significant deviation of the shape of the macromolecule from spherical.

4. The deviation from the spherical shape with increasing temperature is also confirmed by the different growth of the components f_{44} and f_{33}, which is expressed in the difference from zero difference ($f_{44} - f_{33}$), at $\alpha \sim 90°$, which can also be used as a criterion for the nonspherical shape of scattering particles.

5. An increase in temperature introduces changes not only in the shape of the particles, but also in their geometric dimensions, as evidenced by the change in absolute value of the components $f_{12}(f_{21})$ and the components f_{33} and f_{44}. Thus, one of the criteria for the particle size can be the value of the angle a, at which the component $f_{33} = 0$. An increase in the parameter of scattering particles leads to a shift of the zero value f_{33} (f_{44}) towards large scattering angles. Based on the data obtained, it can be assumed that with an increase in temperature, the particle size first increases (transition to a helical conformation), and then decreases with an increase in the number of irreversibly denatured biopolymer molecules. In addition, the biopolymer solution brought to coagulation becomes cloudy, which naturally causes a drop in the value of all polarization components of the light scattering matrix.

6. With a tenfold decrease in the concentration of the solution, the sensitivity to changes in the shape and size of macromolecules remained in the component f_{12} (f_{21}) and, to a lesser extent, in. It is difficult to judge the magnitude of the change in shape, but the tendency for these components to fall in absolute value remains.

7. In addition, when conducting research, it is necessary to take into account the time dependence. The denaturation process does not occur instantly with an increase in temperature, but has a certain time dependence. Therefore, the change in the components f_{12} (f_{21}) in absolute value with a short observation time is insignificant.

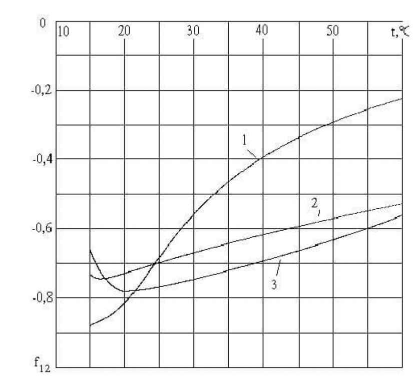

Fig. 3. Temperature dependence the component f_{12} of LSM of fish mucus at various concentrations in a buffer solution (curve 4 -1 g / ml), (curve 2-2 g / ml) and (curve 1-3 g / ml) at α = 90 °.

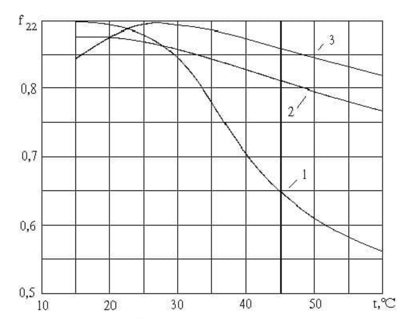

Fig. 4. Temperature dependence the component f_{22} of LSM of fish mucus at various concentrations in a buffer solution (curve 4 -1 g / ml), (curve 2-2 g / ml) and (curve 1-3 g / ml) at α = 90 °.

The objects under study were placed in the center of the goniometric unit in a multifaceted cuvette with immersion liquid (n = 1.49) to reduce the air-lining-cover glass interface. The measurements were carried out in the range of scattering angles 5° ÷ 70° with a discreteness of 5°.

According to the results obtained for fish scales, LSM is characteristic of chaotically located non-spherical, isotropically absorbing objects:

$$\begin{vmatrix} 1 & f12 & 0 & 0 \\ f21 & f22 & 0 & 0 \\ 0 & 0 & f33 & -f34 \\ 0 & 0 & f43 & f44 \end{vmatrix}$$

Analysis of the results obtained allows us to note the following:

1. The smoothness of the angular dependences f_{ik} in the investigated range of scattering angles α is due to the sequence of this system.
2. Components $f_{22} = F_{22} / F_{11}$ the ratio of the intensity of polarized radiation in the scattered light in the total intensity. The depolarization value $\Delta = 1 - f_{22}$ is a measure of the deviation of scattering particles from a spherical shape, since for a system of spherical particles of any size f_{22}, its MSL is identically equal to unity in the entire range of scattering angles, that is, for homogeneous spheres $\Delta = 0$, in our case Δ differs from 0 by 0.3 and reaches its maximum value in the range of scattering angles $\alpha = 95°$. Obviously, the main parameter that determines the deviations from the spherical shape will be the maximum value of Δ.
3. Components $f_{12} = F_{12} / F_{11}$ correspond to the degree of linear polarization for singly scattered unpolarized incident radiation. According to the graphical dependences, a positive f_{12} value in the region of small scattering angles can be caused by the absorption of radiation by cell nuclei.
4. $f_{43} = -f_{34}$ for spherical particles corresponds to the degree of ellipticity of the radiation scattered by the system. This component senses the shape of scattering centers to a lesser extent than f_{22}.
5. The components f_{33}, f_{44} for scattering by homogeneous spheres are equal to each other in the entire range of scattering angles α.
6. In the range of angles $\alpha > 90°$, the difference $f_{44} - f_{33}$ can serve as a criterion for the asphericity of structural elements.

The component is f_{33}, and the exact value of the scattering angle where $f_{33} = 0$ can serve as a dimensional parameter. An increase in the size parameter of scattering particles leads to a shift of the zero value of f_{33} towards larger α.

Small values of the f_{12} component indicate significant sizes of structural elements.

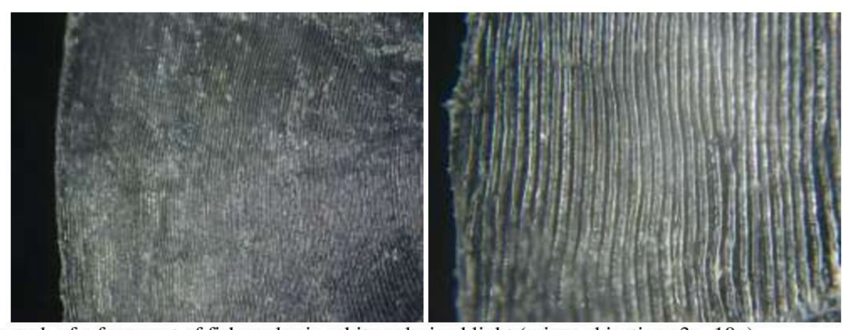

Fig. 5. Micrograph of a fragment of fish scales in white polarized light (micro objectives 3x, 10x)

Studies of the scattering of polarized light from a fragment of fish scales (Fig.6-9) Showed that their MRS has the following structure:

1. At При $\Theta = 0°$, that is, the well-equipped structures are located parallel to the horizontal plane of scattering (the scattering medium has a horizontal plane of symmetry), the MSL of the cut has the form:

$$\begin{vmatrix} 1 & 0 & 0 & 0 \\ 0 & f22 & 0 & 0 \\ 0 & 0 & f33 & -f34 \\ 0 & 0 & f43 & f44 \end{vmatrix}$$

The presented MLS is typical for a medium with chaotically oriented dispersed asymmetric particles.

2. When $\Theta = 90°$, that is, the well-equipped structures of fish scales are located perpendicular to the horizontal scattering plane, the MLS has the same form as in the case when $\Theta = 0°$, except for the opposite sign of f_{34} and f_{43}.

The course of the angular dependences of the nonzero components of the obtained MRS is shown in Fig. 6-8.

When studying the diffraction field of oriented fragments of fish scales, made in along ordered structures, the ratio of polarized components at orthogonal orientations ordered with respect to the scattering plane, namely H_H^{90} / H_H^0, V_V^{90} / V_V^0 indicate a high sensitivity of such characteristics to the orientation of ordered structures. So the ratio of the intensity of polarized radiation with orthogonal arrangements of samples for well-oriented structures is about 12, while for chaotically oriented about 2 Fig. 9.

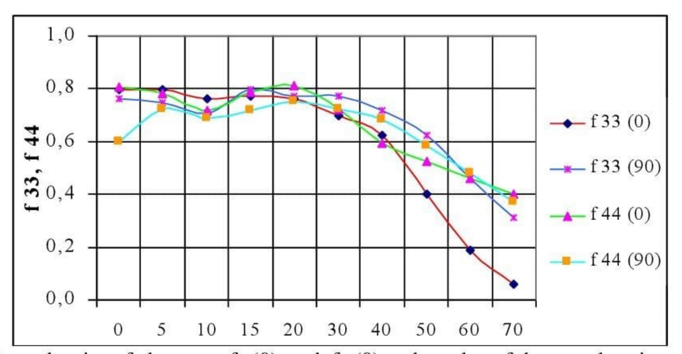

Fig. 6. Angular dependencies of elements: $f_{33}(0)$ and $f_{44}(0)$ at the value of the sample orientation angle $\Theta = 0°$ $f_{33}(90)$ and $f_{44}(90)$ at a sample orientation angle $\Theta = 90°$

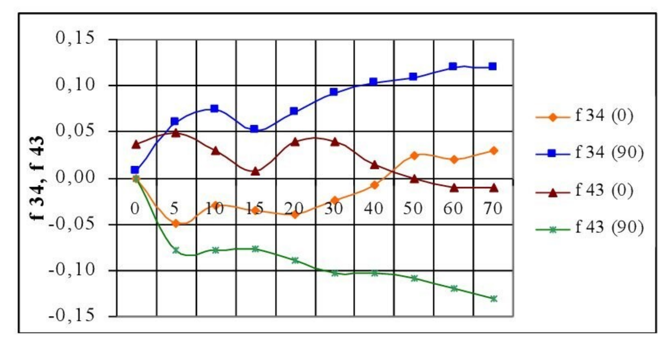

Fig. 7. Angular dependencies of elements: $f_{34}(0)$ and $f_{43}(0)$ at the value of the orientation angle of the sample $\Theta = 0°$ $f_{34}(90)$ and $f_{43}(90)$ at a sample orientation angle $\Theta = 90°$

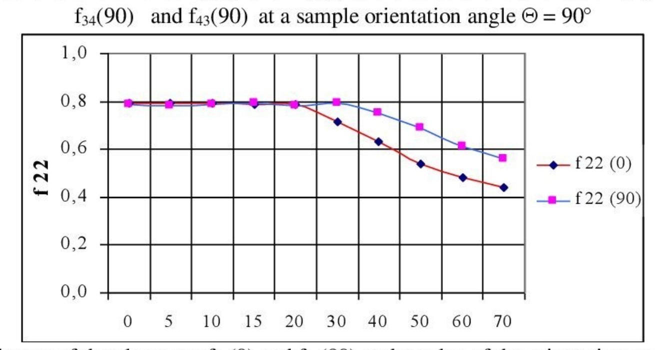

Fig. 8. Angular dependences of the elements: $f_{22}(0)$ and $f_{22}(90)$ at the value of the orientation angle of the sample $\Theta = 0°$ and 90°

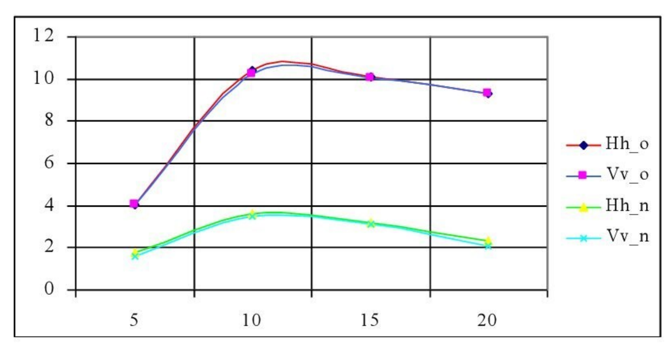

Fig. 9. Ratio of linearly polarized components for ordered structural elements of fish scales (Vv_o i Hh_o sample 2), (Vv_n and Hh_n sample 3)

4. CONCLUSIONS

As a result of an experimental study of the scattering of polarized light from various fragments of fish scales, th following regularities of the angular motion of the MSL elements were found:

1. The smoothness of the angular dependences f_{ik} in the investigated range of scattering angles α is due to th polydispersity of this system.
2. Small values of the f_{12} component indicate significant sizes of ordered structural elements of fish scales and their polydispersity.
3. The value of depolarization $\Delta = |1 - f_{22}|$ reaches its maximum value of 0.6, which indicates a significant deviation of the shape of the scattering centers from spherical. The differences in the angular behavior of the f_{33} and f_{44} components also indicate the deviation of the shape of the scattering centers from spherical.
4. To estimate the dimensional parameter of ordered structural elements, it is advisable to use the value of the scattering angle where $f_{33} = 0$.
5. When the angles of orientation of the ordered structural elements $\Theta = 45°$ with respect to the plane of reference, the components f_{33}, f_{24}, f_{42}, other than zero, indicate the anisotropic properties of the ordered structural elements.
6. To assess the degree of orientation of ordered structural elements, it is advisable to use the ratio, both parallel and perpendicular to the linearly polarized components of the diffraction field by such objects.
4. In the range of scattering angles $\alpha > 90°$, the difference in absolute value $f_{44} - f_{33}$ serves as a criterion for the asphericity of scattering ordered structural elements.
5. Under normal irradiation of ordered structural elements of various degrees of order in a thin layer by linearly polarized light with a polarization azimuth of 0 or 90 with respect to the reference plane.

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